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Role of estrogen signaling in breast carcinoma: implications for cell metabolism

Úloha estrogenní signalizace v regulaci buněčného metabolismu u karcinomu prsu

Bachelor's thesis

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Prehlásenie

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Podpis

Poďakovanie

Rada by som poďakovala RNDr. Kataríne Smolkovej, Ph.D za ochotu, pomoc a cenné rady pri spracovaní bakalárskej práce.

Abstract

Cellular transformation leads to rapid cell proliferation that causes a global disease called cancer. Breast cancer is the second most frequent group that affects primarily women. Disease progression is stimulated by the female sex hormone estrogen. This hormone affects cells via estrogen receptor signalization, for example, by changes in proliferation, angiogenesis, apoptosis, and differentiation. The estrogen signaling pathway also alters breast cancer cell metabolism. The inhibition of estrogen signalization is commonly used in cancer treatment. Nonetheless, some tumors show resistance to the treatment and increase the need for new targets of therapy which can be found among the changes in breast cancer cells metabolism. This thesis introduces more closely the topic of breast cancer and the effect of estrogen receptors.

Keywords

breast cancer; estrogen receptor; metabolic reprogramming; hormone treatment

Abstrakt

Bunečná transformácia vedie k nádorovému bujnenie, ktoré spôsobuje globálne ochorenie, nazývané rakovina. Rakovina prsníka predstavuje druhú najviac zastúpenú skupinu postihujúcu najmä ženy. Postup ochorenia je stimulovaný ženským pohlavným hormónom estrogénom. Tento hormón ovplyvňuje bunku cez signalizáciu estrogénového receptoru, napríklad zmenami v proliferácii, angiogenézii, apoptóze a diferenciácii. Táto signálna dráha ovplyvňuje aj metabolizmus buniek rakoviny prsníka. V liečbe sa využíva inhibícia práve estrogénovej signalizácie. Avšak, niektoré tumory sú voči tejto terapii rezistentné a je potrebné hľadať nové ciele terapie, ktorými môžu byť zmeny v metabolizme buniek rakoviny prsníka. Tato práca bližšie zoznamuje s tematikou rakoviny prsníka a vplyvu estrogénového receptoru.

Kľúčové slová

rakovina prsníka; estrogénový receptor; metabolické zmeny; hormonálna liečba

Content

1. Introduction

Cancer is a serious disease endangering the lives of millions of people around the world. Breast cancer belongs to the group of diseases that are caused by cell transformation and rapid proliferation. The most endangered in a population are women, especially in the premenopausal period of life. According to WHO, breast cancer was the second common cancer that causes death with 2,09 million cases worldwide in 2018.

Breast cancer cells differ from normal tissue cells in many ways. These cells are strongly edited to divide above the average limit of growth. This ability leads to a pathological state of cells that may cause metastasis. The metastasis is a process of the cancer cluster cell traveling that enables dispersion through vessels of an organism to distant parts of the body. This cancer cell spreading is the most life-threatening phase of cancer disease.

One of the driving forces of carcinogenesis is a female sex hormone called estrogen. This steroid molecule is important for female body development, regulation of female period cycle and other processes. Estrogen impacts breast cancer formation via estrogen receptors. Estrogen receptor is a protein consisted of six domains. Receptors can be activated via two pathways: ligand-dependent and ligand-independent, respectively. As a result of these two pathways, the receptor has several possible signaling cascades that affect cell functioning.

The characteristic feature of cancer cells is their altered metabolism. Breast cancer cell energetics is also influenced by estrogen signaling cascade. This way the cancer cell gains the advantage of fast proliferation. Thanks to the specific metabolic alterations, cells can be selectively targeted by diverse therapies.

Some breast cancer subtypes are effectively treated by focusing on estrogen receptor signal inhibition. The treatment is called hormone therapy. This thesis summarizes the knowledge about the function of estrogen signaling in breast cancer cells and its influence on cells, its metabolism and the role of this concrete signaling in hormonal treatment and resistance.

2. Cell transformation

Cancer, as a systemic disease, originates by neoplastic transformation within a single cell; under some conditions, normal cells can undertake processes leading to transformation into neoplastic cells. The process of carcinogenesis includes several sequential events[1]. Several neoplastic cells survive and proliferate past normal cells and create a foundation for malignancy[2]. Combination of several genomic changes lead to uncontrolled cell growth, typically gain-of-function mutation of proto-oncogenes into oncogenes (*e.g. H-RAS*, *ErbB2*, *PI3KCA*, *MYC*) and inactivation of tumor suppressor genes (*e.g. RB*, *CHK2, PTEN, TP53*, *RAD50*)[3].

Transformed cells attain characteristic biological features which enable the cancer progression, called "hallmarks of cancer"[2] (Fig.1.). Alterations in a genome may lead to the formation of "mutant" cells. Unlike normal cells, the proliferation of which is strictly under control, cancer cells deregulate proteins involved in the cell cycle. The activation of growth-stimulatory signals happens through the induction of proliferative signals, the release of mitogenic signals, and attenuation of cell growth down-regulators. Proteins involved in the process are basically tumor suppressor genes and they inhibit cell proliferation. For the subsequent growth, the tumor must avoid the natural cell attribute called contact inhibition. Contact inhibition means that proliferation is repressed to prevent an abnormal number of cells in the limited space and dense conditions.

Another hallmark of transformed cells is the ability to avoid cell death. Proliferation can be accelerated to that extent an error in the cell cycle occurs and it may lead to apoptosis. Cell death can also be invoked from the outside. It is crucial for the cancer cell to inhibit the apoptotic pathway in order to survive and proliferate.

Next, normal cells are programmed to avoid cell damage and to protect the organism by having a limited amount of growth and division cycles (Hayflick limit). Cancer cells must be immortalized which means that they must escape the Hayflick limit and enhance the replicative potential. With increasing cell numbers, nutritional demands are also increased, so growing tumor mass is forced to develop the new vasculatures in order to supply the cells with nutrients[2].

Immune system (IS) cells also play a role in cancer progression. Tumors are infiltrated by immune cells. These cells provide support for tumor survival. On the other hand, If the cell is transformed properly, there is always a risk of detection by IS which normally eliminate not fitting cells[2].

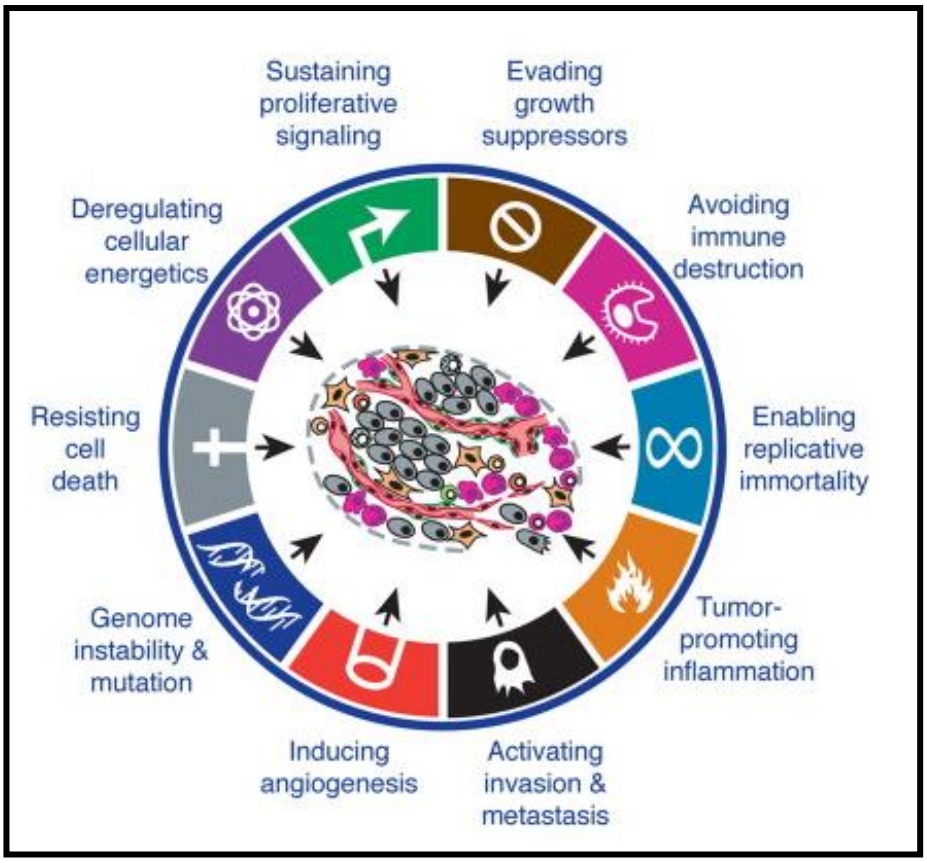

Fig.1. The hallmarks of cancer. The circle illustrates the unique abilities of cancer cells important to the progression of carcinogenesis. Edited from [2].

2.1 Activation of invasion and metastasis

The most dangerous and relevant hallmark of cancer cells is the ability to form a metastasis[2]. During the initial stages of cancer the primary tumor is formed; further stages include the activation of the metastatic cascade which consists of invasion, intravasation, the arrest of cancer cells, extravasation, and neovascularization[4]. Metastasis represents an increased risk for the patients; spreading cells to other parts of the body and formation of metastases brings poorer outcome and increased risk of death.

The formation of metastasis is crucial for survival. This is how the tumor avoids metabolic and proliferative limitations[5]. There is also a correlation among metabolic flexibility and survival of metastases in distant tissue[6].

Metastatic process is described by *clonal selection hypothesis*, where a subpopulation of the cells in primary tumor forms a secondary tumor (Fig.2A)[7]. The theory is challenged nowadays by cancer stem cell models, where the same cell clones of pluripotent cells are responsible for primary and secondary tumors (Fig.2B)[8].

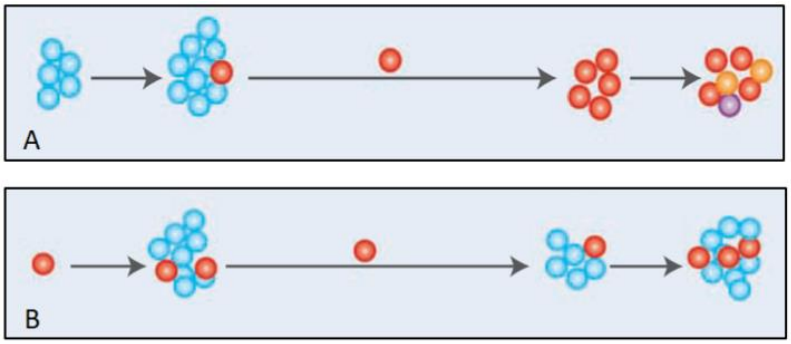

Fig.2A. Clonal selection of the metastatic progression. **Fig.2B.** Stem cell model of metastatic progression. Edited from [7].

Invasion-metastatic cascade is a sequence of events happening in exact order. It starts with the local invasion through the extracellular matrix. Then, progressing cells penetrate blood or lymph vessels in a process called intravasation and are carried through the lymphatic or hematogenous system. Such cells are called circulating tumor cells (CTCs)[9]. The next step is the escape from the lumina of vessels to the parenchyma of distant tissue. The opposite action of intravasation is called extravasation. Before the arrival of the interspersed tumor cells from the primary site to neighboring tissue, it is crucial to pre-form the pre-metastatic niche for establishing metastasis[10]. The successful clones now create small nodes that grow over time[2].

The process of metastasis also includes changes in cells potency. The epithelial-mesenchymal transition (EMT) is the initial step in the metastatic process. In the process of EMT epithelial phenotype is lost, the cell with the mesenchymal characteristics arise and consequently colonize distant tissue (Fig.3.). This cell can migrate[11] due to a modification in the expression of adhesion molecules *e.g.* in E-cadherin, EpCAM, keratin-14[6]. The reverse process of EMT, called mesenchymal-epithelial transformation (MET), is also necessary for metastasis in the matter of formation of a new tumor cluster[11]. Endothelial-mesenchymal transition (EndMT) is the transformation of vascular endothelial cells to mesenchymal cells[9]. The cell surface is altered in a way independent on the contact with other cells and allows its penetration through the basal membrane (BM). Therefore, intravasation and extravasation are possible[6].

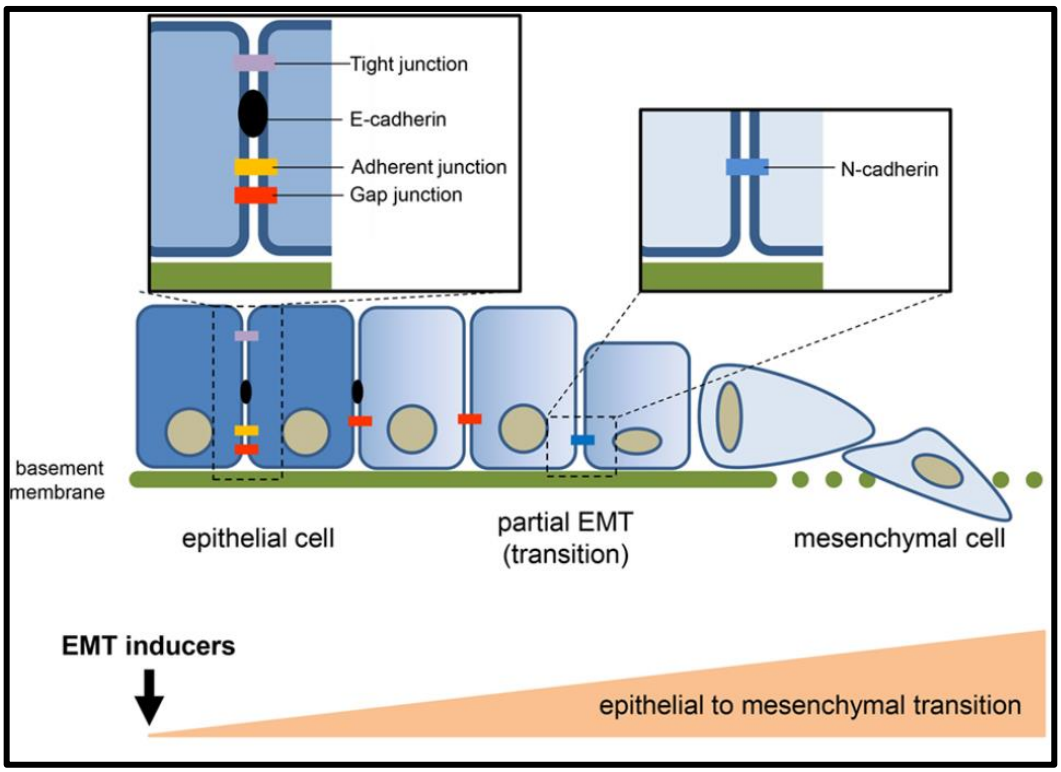

Fig.3. The process of EMT. The transformed epithelial cell gradually loses contact with the surrounding cells and cell polarity. These cells have changed the shape and surface, so the adhesion is adjusted. Finally, this cell is transformed into a mesenchymal cell. Edited from [96].

2.2 Metabolic changes in cancer cells

Altered cancer metabolism is one of the hallmarks of cancer cells. Cancer cells have a considerably higher requirement for energy than non-transformed cells[5]. Because of higher rates of proliferation (essential for the formation of tumor mass) cell growth requires biosynthetic precursor and induce changes of cell energetic pathways, such as glycolysis, glutaminolysis, β-oxidation, de-novo fatty acid (FA) synthesis, serine synthesis, and others[2].

Alteration of metabolic pathways in cancer cells supports energy production. The main source of energy for the cell, the ATP molecules is preferably acquired from the aerobic glycolysis even if oxygen is present[5,12]; lactate is produced under aerobic conditions instead of channeling pyruvate to mitochondrial oxidation in Krebs cycle[13]. Incomplete oxidation of glucose into lactate offers cell 18 times less ATP/mol glucose than glycolysis with citric acid cycle and respiratory chain included, but it is about 100 times faster regarding the duration of both reaction pathways and that gives advantage to the transformed cells[5]. This metabolism generates an acidic environment which might support extracellular matrix (ECM) degradation and invasive phenotype[5].

Moreover, alteration of metabolic pathways also supports cell growth by generating metabolic precursors for anabolic processes. The aerobic glycolysis provides important growth profit as a carbon source along with the energy source (Fig.3.). The building blocks acquired via glycolysis are important in highly proliferative cells and they are used as a base for nucleic acid synthesis and amino acid production[5]. Increase in glycolysis causes increased levels of intermediates such as glucose-6-phosphate that favors pentose phosphate pathway that generates reducing equivalents (NADPH) subsequently used in both redox homeostasis maintenance and FA biosynthesis. 3-phosphoglycerate produced in glycolysis is a precursor for the serine synthesis pathway and commonly used in cancer cells to support proliferation[5].

In a highly proliferative stage, the cells increase glycolytic and decrease oxidative metabolism. After, the highly proliferating cells become to be denser and more hypoxic the hypoxia-inducible factor-1 (HIF1) supplies the source of oxygen and nutrition by angiogenesis[14]. Then migration and metastasis could take place. These cells are exposed to oxidative stress and hypoxia that let them to stemness, angiogenesis and invasiveness, and metastatic potential. These cells are characterized by increased mitochondrial biogenesis of ATP. The metastatic capabilities of BC cells are ensured by PGC-1α-induced oxidative phosphorylation (OXPHOS) metabolism that is essential for tumor progression. In the phase of increased stem cell properties heterogeneity in bioenergetic properties is found. Some cells rely on glycolytic metabolism and some prefer OXPHOS metabolism[14].

Glutaminolysis is another altered metabolic pathway in cancer cells; 2-oxoglutarate is anaplerotically formed from glutamine and supports mitochondrial metabolism. Glutamine transporter (ASCT2) and glutaminase (GLS) are commonly upregulated enzymes in cancer cells leading to induction of glutaminolysis[15]. Krebs cycle of cancer cells is also altered; citrate is exported from mitochondria to support de-novo FA synthesis. We call this feature “truncated Krebs cycle”. 2-oxoglutarate is compensated by glutaminolysis. Glutamine is also used as an antioxidant in cells[14].

Among other mitochondrial alterations, cancer cells are often dependent on pyruvate carboxylase (PC) enzymes, which supports anaplerosis of both pyruvate carboxylation and glutaminolysis. For progression and surveillance of invasive and metastatic cells, it is important to maintain pyruvate cycling. The major TCA (tricarboxylic acid cycle) intermediates are exploited in metabolic pathways as biosynthesis of lipids, nucleic acids and some amino acids that correlates with cell proliferation. There is a correlation between PC and tumor size and stage[13].

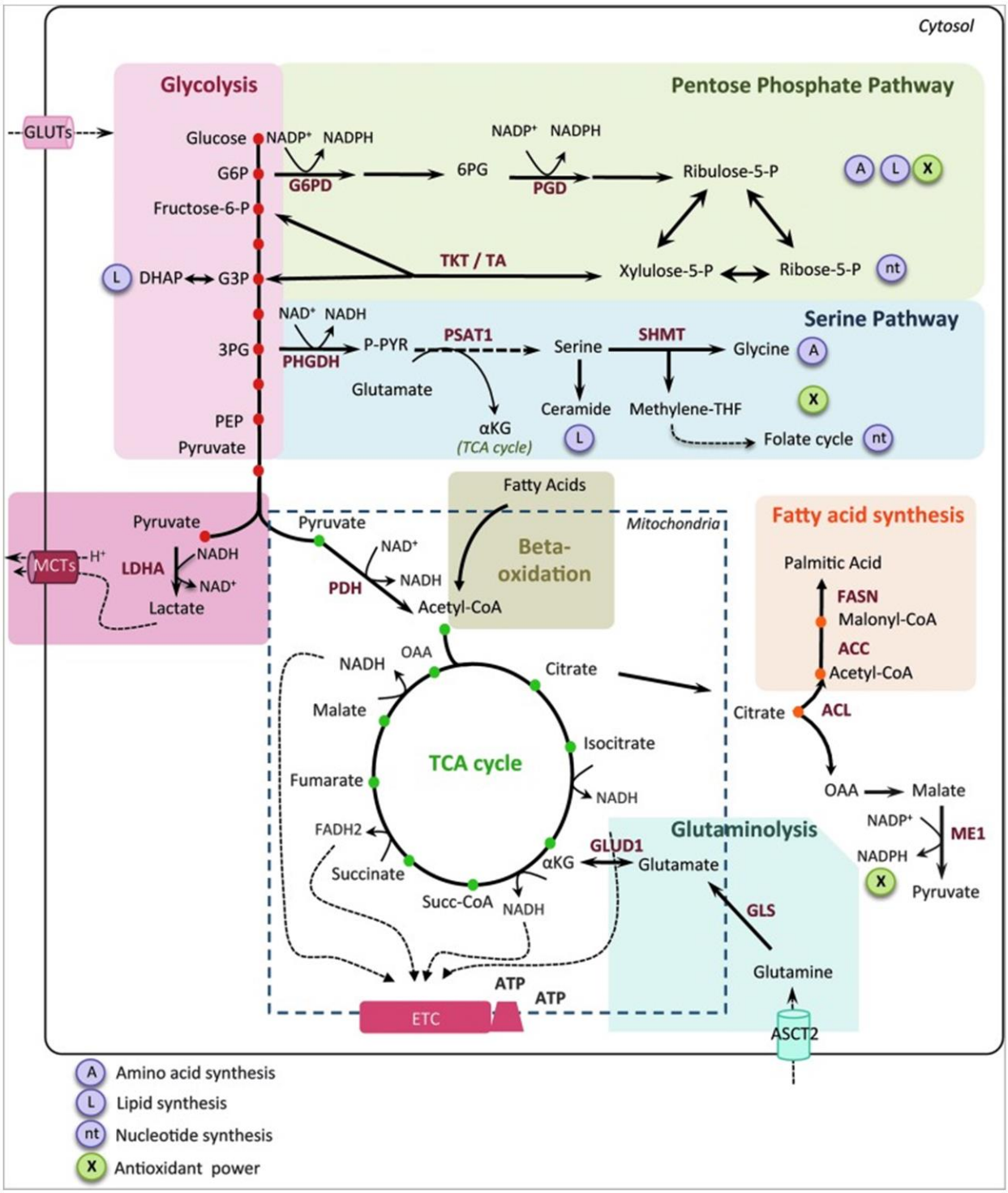

Fig.4. Summary illustration of metabolic alterations in cancer cells.
Taken from [14].

3. Breast cancer

Breast cancer (BC) is a very common heterogeneous group of malignant diseases. In developed countries, carcinoma of breast belongs to the most frequent cause of death among women (there is variation between ethnic groups)[16]. The risk of breast cancer significantly increases during life, mostly in premenopausal women. This hormone-dependent disease prevails mainly in the female population, still, men could also be in danger[17]. Typical metastatic sites of breast cancer are the lung, liver, brain, or bone[10].

Every year 2.1 million women develop the disease worldwide[18]. In 2016 the incidence of BC in the Czech Republic was more than 130 cases per 100,000 of women, *i.e.* more than 7000 of newly diagnosed cases per year[19]. The percentage of 5-year survival after cancer for women with invasive BC is 90 %, 10-year survival is about 83 %. The 5-year survival for cancer located only in the breast is 99 %, for the disease that is spread to lymph node the survival rate is lowered to 85 % and in case of the metastatic disease the survival rate is only 27 %[20].

3.1 Breast cancer classification

We distinguish several groups of BC from a clinicopathological and genetic point of view[21].

Tumor progression of the breast carcinoma could be classified in terms of morphological features as ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC)[10]. The tissue of origin within the breast (the lobules or the ducts) also determines carcinoma type; the preinvasive *in situ* carcinomas are called depending on the origin as ductal carcinoma *in situ* (DCIS) or lobular carcinoma *in situ* (LCIS)[22]. DCIS phase of the mammary tumor is considered premalignant. Otherwise, the IDC stage tumor is an invasive phase, which includes migration to distant tissue. The main difference between these two stages is the loss of myoepithelial layer and BM in IDC[21]. Breast cancer progression is depicted in Fig. 5.

By histological features such as the extent or spread, BC is classified by "Nottingham Grading System" into grades. Grade 1, the low-grade carcinoma is characterized as a well-differentiated tumor. Tumors that belongs to Grade 2 are moderately differentiated and are the intermediate grade carcinoma. And finally, Grade 3 are cancers with high grade, and these tumors are poorly differentiated[23,24].

Another clinical classification of BC stage is the TNM system. T stands for the tumor size and location; it could be in range 0-4 when T0 is tumor free, and T1-4 depends on tumor measure and range. N means node invasion and consist of 4 stages. N0-cancer cells did not invade lymph nodes, N1-3 nodes are invaded, 2 4-9, N3-more than 10 nodes. And finally, M is a measure of metastasis. M0 no metastatic cancer, M1 evidence of metastasis in a distant part of the body. Combining of T, N, M classification determines the stage of BC. There are 5 stages, 0-IV. 0 is known as DCIS (noninvasive carcinoma in situ) and stages I-IV are invasive cancers[25].

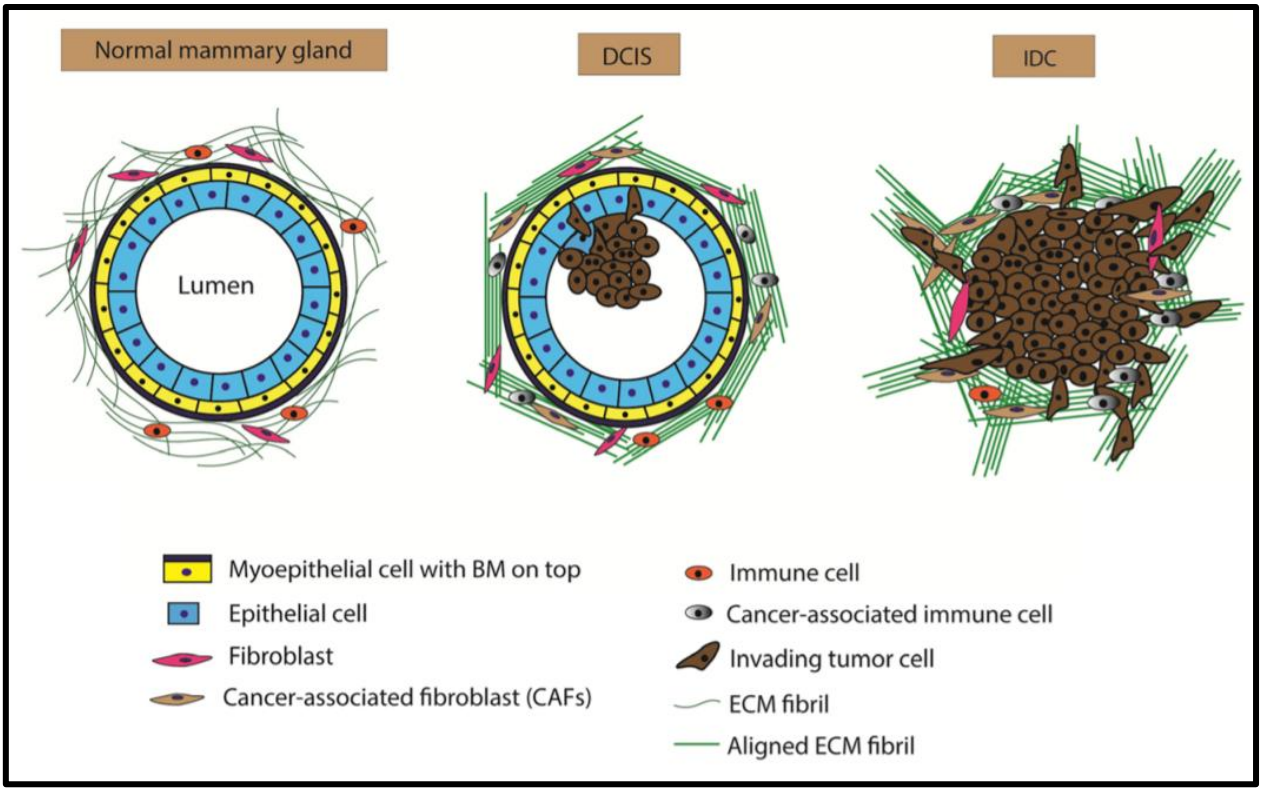

Fig. 5. The normal mammary gland is characterized as well-organized layers of the cells piled up on each other. These layered cells are surrounded by randomly organized fibrillar collagen. There are also other cells needed for mammary homeostasis. After the unregulated proliferation of the epithelial cells in DCIS stage of BC, the cancer cells aggregate in the lumen and other changes of the tissue occur in the area of the mammary gland. After the lumen is almost completely full of transformed cells, the surrounding tissue is organized and represents the path for cancer cell transport. The cancer cells grow through the myoepithelial layer and BM. Edited from [21].

The molecular classification is based on differences in expression of hormone receptors[26]. According to this categorization, we distinguish less aggressive luminal-like that have two forms luminal A form and luminal B, HER2+, basal-like[10], claudin-low(CL)[27] and normal-like breast carcinomas[28].

Luminal A subgroup was characterized as a type with the highest expression of genes that mediate the effect of estrogen and the low expression of genes managing proliferation. Patients suffering from this type have the most positive clinical behavior[29].

Another luminal breast cancer type, luminal B tumors are characterized with lower levels of receptors for estrogen (ER) and progesterone (PR) than luminal A subtype, higher levels of proliferation cluster protein M KI67 and cell cycle-associated proteins (CCNB1 and MYBL2) and increased expression of growth factor receptor genes[30].

ERBB2/HER2+ group is known by overexpression of HER2, low expression of ER and associated genes[29,31].

A basal-like subtype is also known as triple-negative breast cancer (TNBC). It is the heterogeneous group of tumors that have downregulated the expression of ER, PR, and HER2[26]. These tumors also are BRCA1 mutation carriers, have aggressive feature including high proliferative capacity, high histologic grade, and frequent TP53 mutations. Along with HER2+ are considered as more aggressive subtypes with poor clinical outcome[31].

CL subgroup has the most enriched stem cell-like features among others followed by TNBC. The expression of PR and ER is higher than in TNBC, although low. Also, the P53 pathway is downregulated[27]. CL tumors lack in tight junction proteins (claudin 3, E-cadherin), downregulated the expression of luminal markers and upregulation of mesenchymal markers[27].

And finally, normal-like subtype has similar features as normal breast tissue[28].

3.2 Genetic background of breast carcinoma

Diversity among subtypes of BC is characterized by differences in genetic alterations leading to specific tumor phenotype. To achieve this, cells must accumulate genomic alterations[21]. For instance, ER+ and HER2+ subtypes often carry some missense mutations; on the other hand, TNBC tumor has an elevated amount of nonsense, frameshift, and complex mutations. HER2+ and TNBC are enriched in the genomic rearrangements and copy number alterations[21].

The most common source of abnormal growth is genome instability. Mutations of *BRCA1* are responsible for the hereditary form of breast cancer. The risk of BC is increase due to the mutation in one allele of this gene[32].

BRCA1 is a tumor suppressor gene which is normally responsible for maintaining genomic stability, DNA repair, cell cycle, and other processes[33]. BRCA1 protein is activated after the double-strand break of DNA. As a result, BRCA1 is phosphorylated by ataxia-telangiectasia mutated (ATM) kinase and the initiation of DNA repair through a sequence of actions proceeds. Phosphorylated BRCA1 recruit Rad50-Mre11-NBS1 complex that binds to DNA. Also, an inactive complex BRCA2-Rad51 is activated and Rad51 covers single strand sections of DNA. Altogether, these processes enable homologous recombination (HR)[34](Fig.6.).

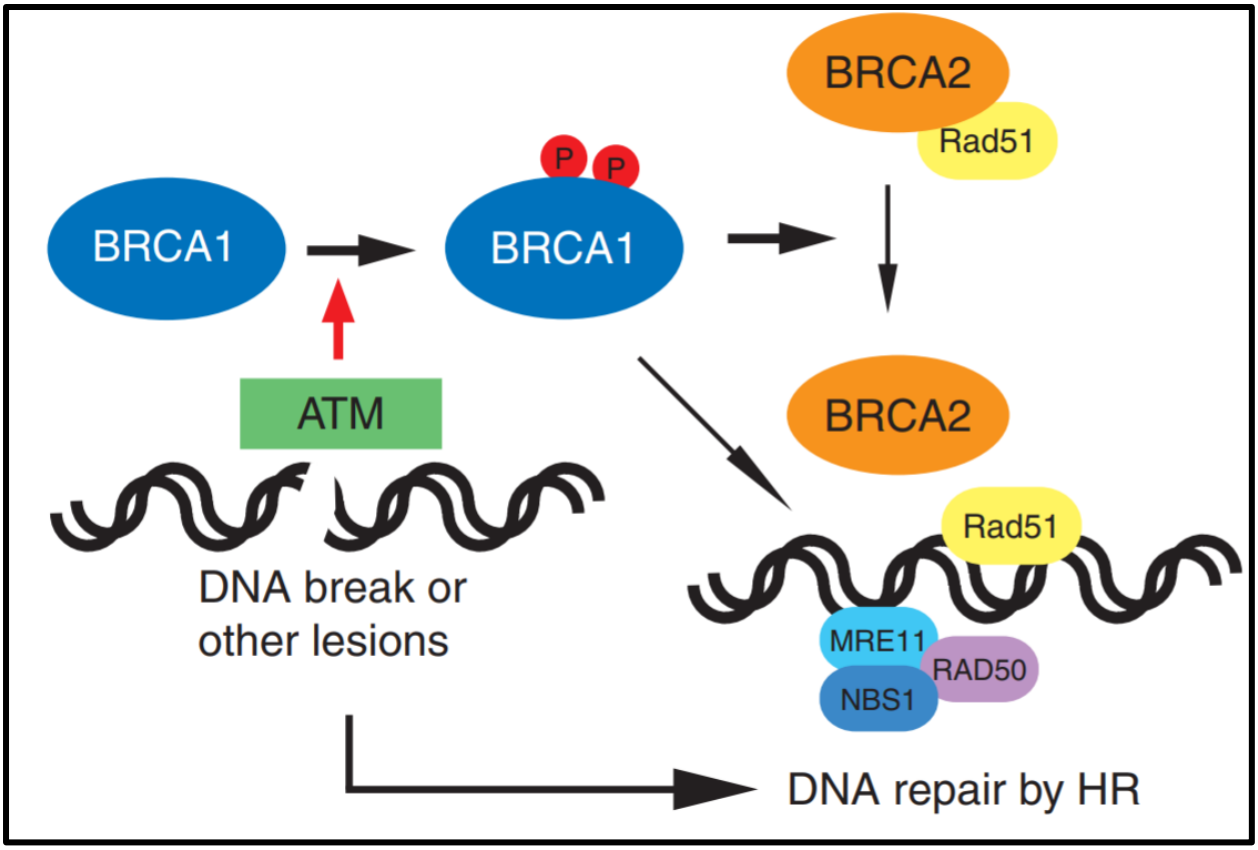

Fig.6. Double strand-strand break repair BRCA proteins mechanism. Taken from [34].

Other typical oncogenes and tumor suppressor genes responsible for breast carcinoma are, for example, in the alleles of the *TP53* gene that encodes the tumor-suppressor protein p53 that interacts with RB1 protein. Their cooperation participates in the regulation of the cell cycle, cell repair, and apoptosis.

EGFR and *ERBB2* (Her-2/neu) gene encode an epidermal growth factor receptor, a cell surface protein called ErbB2. Its role is in cell adhesion, specialization, and motility of the cell. Overexpression of the gene brings many receptors to the cell surface that make a strong signal for dividing and rapid cell growth. It increases the risk of BC and metastasis.

Also, *CDH1* gene codes E-cadherin, the adhesion glycoprotein that is considered as a tumor suppressor in BC. Reduced expression of this transmembrane molecule is related to the metastatic transformation due to increased cell motility[35]. Furthermore, low DNA repair capacity (DRC) can be found among all types of BC and represents a risk of developing the disease, the lowest DRC is found in TNBC subtype[36].

Alterations in these and other genes represent the risk of development of BC. The most commonly mutated genes in breast cancer (over 4 % of frequency) are presented in Fig.7.

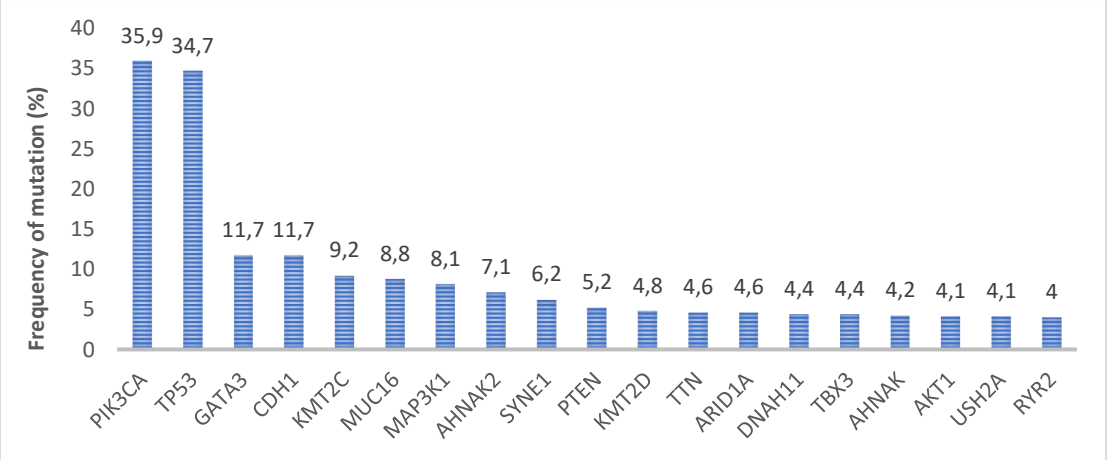

Fig.7. Graph representing mutations in BC cells. Mutated genes in breast carcinoma (over 4 % of frequency) generated by cBIOPORTAL (6,100 samples with mutation data).

3.3 Estrogen receptor

Estrogen is a steroid hormone which originates from cholesterol molecule synthesized from LDL-cholesterol. The main estrogens in the human body are estradiol, estrone, and estriol[37]. Organs that synthesizes estrogens are ovary, placenta, testes and adrenal cortex. Estrogen is needed for evolving secondary sex features and has an impact to almost all organs and tissue of a body including plasma lipid profiles, concentration of coagulator factors, insulin and glucose levels in the blood and nitric oxide synthesis.

ER belongs to a group of nuclear receptors. Estrogen has a vast influence on organism *e.g.* development, migration, proliferation, and survival of targeted cells[32]. Pleiotropic action of estrogen induces changes in almost all body tissue. This action is mediated by estrogen receptors[38].

3.3.1 Structure of estrogen receptor

The cellular localization of ERs is at the nucleus as well as at the plasmatic membrane/cytoplasm[33,40]. The membrane ER has the same structure as the nuclear ER. Localization to the plasma membrane is enabled due to the posttranslational modification of the receptor, the palmitoylation, which anchors receptor to the membrane, as palmitoyl residue increases the hydrophobicity of the protein[32].

The structure of ERα is illustrated in Fig.8. ERs consist of the six functional domains marked A-F. The A domain is located at the N-terminal end along with the B domain and is hypervariable among nuclear receptors. Its function is not clearly understood. B domain contains activation function 1 (AF-1)[38]. AF-1 mediates both ligand-independent and dependent responses vary in type of cell, promotor[38,41] and ligand-specific manner. ERα contains serine residues that can be phosphorylated under specific conditions by several intracellular kinases. This action is regulated by growth factors such as epidermal growth factor,

insulin-like growth factor or fibroblast growth factor. On the other hand, the AF-1 domain interacts with coactivators. AF-1 activates after the dimerization, DNA binding or contact with other proteins.

The domains E and F are localized at the C-terminal end of the receptor. The E domain is known as a ligand binding domain (LBD). This large and complex region with a ligand-dependent activation function 2 (AF-2) is organized to the shape of the hydrophobic pocket for the ligand[38]. The AF-2 region binds coregulators as well, contains the nuclear localization signal (NLS) and is responsible for the dimerization[41]. The F domain may modulate the recruitment of some partners of ERα and defines the balance of the agonist/antagonist for different ligands, mostly antiestrogens.

Two zinc fingers at the domain C called a DNA binding domain (DBD), ensures the receptor binding to an estrogen responsive element (ERE) in the DNA sequence. This domain is the most conserved one. The zinc fingers are formed by tetrahedral coordination of two zinc ions with four cysteine residues. The first zinc finger lodges to the major groove of DNA. EREs, the sequence where zinc finger binds, is 13 bp consensus sequence GGTCAnnnTGACC, the inverted palindrome. It demonstrates the symmetrical binding of two monomers of ER dimer. The second zinc finger plays a role in creating the correct dimerization by recognizing proper spacing among the palindromic DNA element. The C domain is localized at the central region of the protein along with the D domain that represents a hinge area. The fragile region allows conformational changes of the protein during its interactions. The second NLS is located here and plays a role in crosstalk with transcription factors such as Sp1. Various posttranslational modifications modulating the activity of the receptor are subjected in the same region[38].

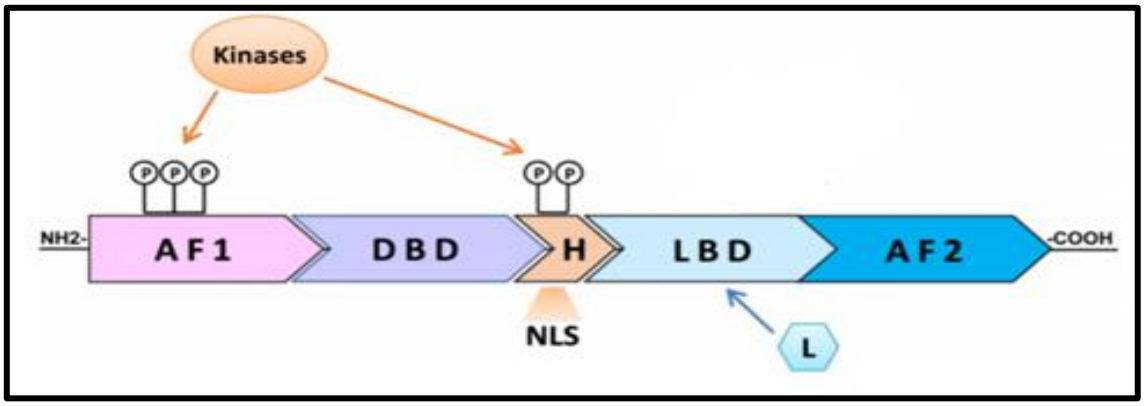

Fig.8. ERα organization

The protein consists of functionally diverse domains: AF-1 with posttranslational modification sites for phosphorylation, DBD, hinge region (H) with another phosphorylation sites and NLS, LBD where the ligand (L), such as estrogen, binds and AF-2 that is ligand dependent. There are several cellular kinases responsible for posttranslational modifications. Edited from [97].

3.3.2 Intracellular estrogen signaling

The pleiotropic effect of estrogen signaling is mediated by ER which has estrogens as the agonist[41]. The cells express two different isomer receptors, called ERα and ERβ[32].

ER has several pathways of signaling. The first one, genomic, is slow one (the effect comes within hours). The estrogen receptors have nuclear localization[39,42]. The genomic pathway starts with the estrogen binding to the ER and its transition to the nucleus[42].

LBD is in an "open conformation" while no ligand is bound. After the ligand binds to the receptor hydrophobic pocket, several remodeling processes take place. These changes modify the protein to the more compact state and the signaling may start[38]. After estradiol binding, two receptors dimerize and conform homodimer or heterodimer (αα, ββ, αβ)[32]. The genomic signaling pathway continues with the receptor translocation to the nucleus[38].

The action of ER in the nucleus has two different pathways. The classical one is by direct protein binding to DNA, to specific EREs sequences in the promoter regions of the targeted gene. The liganded ER recruits transcriptional co-factors and components of RNA polymerase II complex and altogether regulate the transcription of the targeted genes[38,41].

An alternative pathway runs without receptor direct binding to DNA. The liganded ER interacts by the protein-protein interaction with other transcription factors at promotors including Sp-1 and AP-1 proteins, NfκB (Nuclear factor κB), GATA-1[41]. Some of the estrogen-dependent genes contain nuclear hormone receptor SF1 and SF2 response elements instead of EREs[32].

The other possible way is nongenomic signaling with a rapid effect in a range from a few seconds to one minute[39,42]. These responses are ensured by ER that are localized at the cellular membrane where the important and fast signaling pathway starts. The steroid hormone signaling cascade is mediated by receptor induction which activates tyrosine kinases, MAPKs (mitogen-activated protein kinases), G-proteins and modulates ion channels opening. Another option for inducing the signals is the allosteric modifications of receptor and/or structural and enzymatic proteins of the cell membrane. The non-genomic pathway also affects transcription by phosphorylation of TFs.

Integration of both nuclear and membrane-localized ER signaling creates unique action. The membrane ER signaling goes through cAMP-dependent protein kinase A (PKA) or MAPK and can affect the nuclear ER actions[32].

There is a correlation between ER and a growth factor (EGF, IGF, and insulin) signaling pathway. The growth factor leads to the phosphorylation of ER at the AF-1 domain[43].

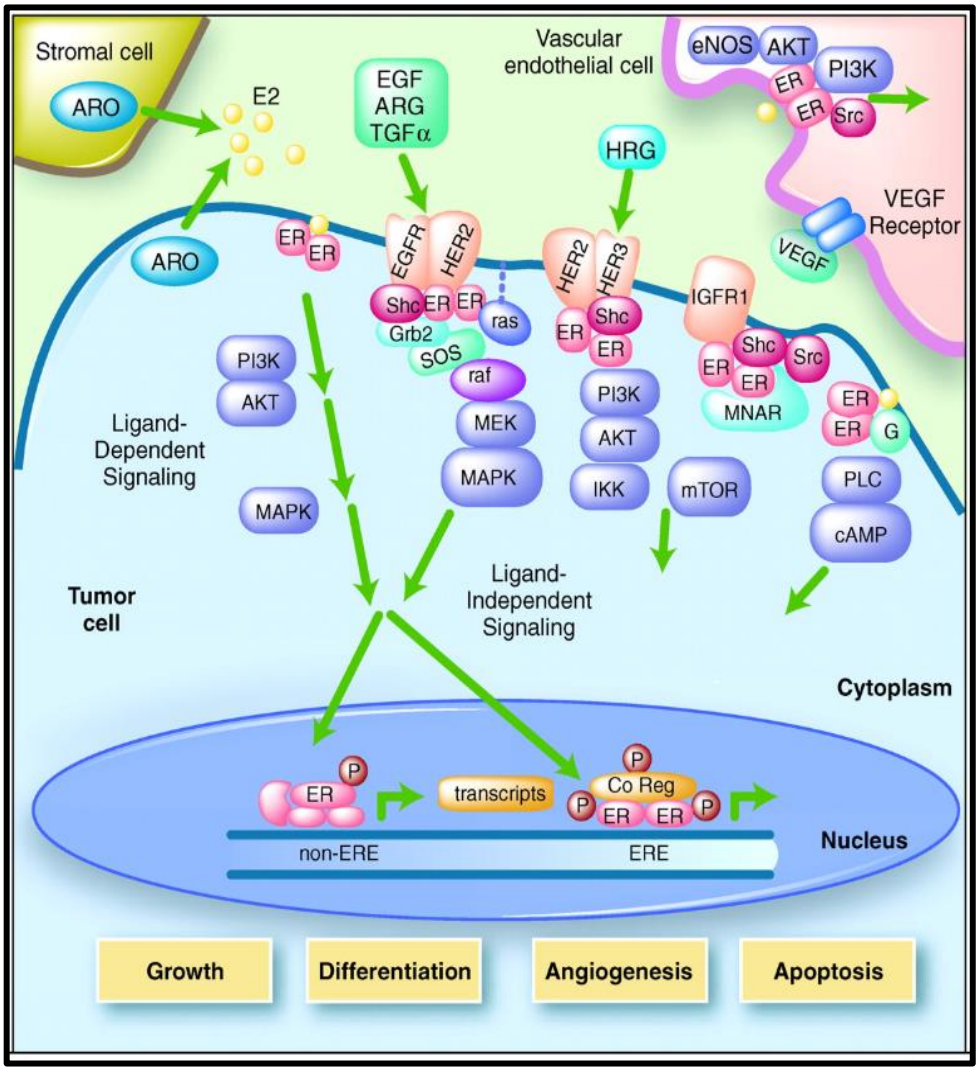

Fig.9. Estrogen receptor (ER) signaling pathways.
Schematic visualization of known ER signaling cascades. The classical pathway of ER action is mediated by estradiol (E2). E2 binds to ER and affects transcription via ER dimerizing and directly binds to ERE on the promoter of the targeted gene. Another ligand-dependent pathway is an alternative genomic pathway. This pathway is ERE-independent, and regulation is done via interactions with other transcription factors (TFs). Another ER action is mediated by interactions with transmembrane growth factor receptors such as EGFR, HER2 and insulin-like growth factor receptor (IGFR1). Their signaling cascades affect cell function. The nongenomic mechanism is mediated by E2 interaction with ER at the cellular membrane that stars the signaling cascade in the cell by activating the cellular kinases that change the function of cellular proteins. Taken from [98].

3.3.3 ERα and ERβ

ER has two isomers that are called ERα and ERβ. ERα is coded by *NR3A1* (also called *ESR1)* gene and ERβ by *NR3A2* (also called *ESR2)*[38]*.* The genes coding these proteins are located on other chromosomes in human genome[32].

The expression and tissue distribution of the isomers differ*.* ERβ is more common in a normal human mammary gland than ERα[44,45]. ERβ is present at high concentrations in the nuclei of the myoepithelial cells. ERβ is also expressed in lymphocytes, stromal cells, and endothelial cells[45].

Contrarily, the ERα is localized mainly in epithelial cells lining ducts and lobules, specifically at the nuclei. ERα expression increases with age and is regulated by PR[45].

ERβ serves as a gatekeeper in cancer biology and slow down the tumor growth and inhibits its progression[44]. ERβ is responsible for the negative regulation of estrogen signaling antagonizes the transcriptional activity mediated via ERα, however, there are few exceptions, *e.g.* proliferative function, when ERβ can also act as an ERα agonist. Therefore, it is considered that ERβ has a bifacial character[44,46]. The activity depends on ERα/ERβ ratio in such tissue[47].

3.3.4 Molecular targets of estrogen signaling

ER pathways induce a wide number of responses that causes changes in almost all the organs and tissue of the body. At the cellular level, proliferation is altered via non-genomic signaling through ERK-related kinases[42]. The non-transcriptional PI3K/Akt (Phosphoinositide 3-kinases/protein kinase B) signaling pathway induced by 17-β-estradiol (E2) results in the increase of proliferation*.* Akt also affects the reduction of antiapoptotic signaling[48]. E2 promotes the human serine proteinase inhibitors serpin PI-9 and influences the cell's response to apoptosis induced by immune system[49]*.*

Another role of estrogen is in the regulation of insulin sensitivity and glucose homeostasis in muscle and adipose tissue cells. In this peculiar case, ERα and ERβ have the opposite effect. ERβ action is diabetogenic by reducing the expression of GLUT4 that maintains glucose homeostasis in a body and its depletion leads to glucose tolerance cutback[47]. Furthermore, E2 affects the expression of an insulin receptor and affects the adipose tissue[50].

The gene transcription of many NOD-like(nucleotide-binding oligomerization domain-like) receptors can be altered by ER depending on the cell type[51].

E2 affects vessels density, structure, organization and stability with mural cell recruitment and affects vessels maturation[52]. The dilation of blood vessels is also estrogen-regulated. Endothelial nitric oxide synthase is stimulated via ERα activation of MAPK pathway. The synthase produces nitric oxide (NO) in the pulmonary endothelial cells and the NO production leads to the blood vessels dilatation.

ERα stimulation of MAPK signaling cascade in other types of cells leads to a different effect. In neuronal cells, the stimulation results in neuroprotection after glutamate excitotoxicity, in bone cells, it is essential for bone formation by regulating osteoblast proliferation and differentiation[42].

3.3.5 Role of estrogen signaling in breast carcinoma

ER represents an advantage for BC lines in several ways. The signaling support, *inter alia*, tumor proliferation and that is why estrogen is considered as a key factor for its growth.

ERα stimulates genes participating in *e.g.,* proliferation, steroid/xenobiotic metabolism, and ion transport. BC cells often depend on E2 in proliferation and cell growth[53].

E2 influence the cell cycle progression via inducing the escape from G1 arrest and pass to the S phase due to the cyclin-dependent kinases that are activated by E2 as well[54]. E2 upregulates expression of the genes for cyclin D1, A2, cdc2, cdc20, and Bub1 that are responsible for the progression of the cell cycle along with other genes that are also regulated by E2 and genes involved in DNA synthesis[53]. ERα induces expression of c-Myc and downregulates an inhibitor for p27 Kip1 the Cdk2 protein whereas ERβ has the opposite effect of their expression. Therefore, ERβ signaling suppresses BC cells proliferation, unlike ERα with the opposite effect[55].

E2 improves cell proliferation and tissue development. E2 regulates cell translational regulation via estrogen-responsive genes of eukaryotic translation initiation factor 3 subunits. Cell proliferation and tissue development are improved. The oncogenes are involved in the growth and/or migration of ER+ BC cells[56].

Normal mammary gland development is also based on increased cell proliferation that requires ER regulation of transcription factors such as HOXC6 and A-Myb. Whereas HOX6 is downregulated in the normal tissue growth, in transformed cells, HOX6 is upregulated via ER cascade. The upregulation gives BC cells the advantage of the cell proliferation support[53].

E2 also downregulates several genes. The downregulation of the genes which code the proteins, known as the cell cycle inhibitors, enhance proliferation and the cell cycle. The direct inhibitors of the cell cycle lowered by E2 are BTG-1, BTG-2, and cyclin G2. The genes coding cytokines and growth factors inhibiting cell proliferation (proteins from TGFβ superfamily) are also decreased by ER pathway[53].

The tumor suppressor p53 is under estrogen action control. Efficient growth of the mammary gland cells is established by suppression of the p53 activity and consequently the growth-inhibitory proteins under p53 control. Additionally, the prolonged exposure to the E2 allows the damaged cells to bypass the p53-regulated mechanisms of DNA repair and cell elimination by p53-dependent apoptosis. This can ultimately result in the gain of the mutation in p53 itself, a key step in the tumor progression resulting in an increased cytotoxic and chemotherapeutic resistance.

The cell cycle phase control, DNA replication, and repair are estrogen-dependent and cohere with the E2 regulation of the gene for transcription, mRNA processing, splicing and transport, ribosome biogenesis, protein folding, and degradation[54].

For better fitness, BC cells must avoid cell elimination and that is secured by ER signaling. E2 dependent cells have better viability due to the antiapoptotic effect[54]. The upregulation of the genes for surviving inhibits apoptosis[53]. E2 induces the expression of Bcl-2 protein, therefore, Bcl-2 and Bax ratio changes.

The ratio serves as a marker for mitochondria contribution and apoptosis. The protein p53 is a positive transcriptional regulator for Bax and negative for Bcl-2. Consequently, E2 decreases the expression of p53 which affects the regulation of other proteins expression. There is a direct interaction between ERα and p53. ERα regulates another protein *mdm2* which is responsible for p53 localization and stability[49].

The tumor growth is supported by another remarkable process. E2 can upregulate its own synthesis by a feed-forward cascade. ER signaling upregulates prostaglandin E synthetase and prostaglandin E receptor type 3 which in consequence increases the synthesis of E2[53].

The invasive and metastatic phase of BC is induced by the ER and PR signaling. These receptors are involved in carcinogenesis where either of the steroid receptors correlates with the expression of NF-κB and tumor necrosis factor alpha (TNF-α). TNF-α is known as a cytokine involved in apoptosis, inflammation, and immunity[57].

E2 is also able to induce the creation of new blood vessels. Without the blood supply, the tumor is limited and cannot continue in the growth, hence, it is highly dependent on the nutrition that the angiogenesis provides. The angiogenic factor TSP-1 is upregulated after the E2 stimulation. E2 regulates the balance between pro- and anti-angiogenic factors and via paracrine interactions between endothelial cells, tumor cells, and other stromal cells increase the tumor angiogenesis and subsequently, the tumor growth. The tumor avoids hypoxia and necrosis by receiving nutrients and oxygen[52].

Hormonal dependent activation of c-Myc leads to the transactivation of the gene for the glucose transporter-1 (GLUT1) independently on the hypoxic conditions in the extracellular microenvironment[55]. The c-Myc activity could be enhanced by E2 action directly and eventually by downregulating an inhibitor of c-Myc[53].

ERβ signaling normally antagonize ERα response in a way that the ERβ inhibits the pro-angiogenic factor PDGFβ leading to the reduction of angiogenesis and consequently the tumor growth is inhibited[58]. ERβ inhibits the metastatic progress via upregulation of the expression of the gene for E-cadherin, a molecule responsible for cell-cell interactions[58]. ERβ signaling inhibits proliferation by repressing c-Myc, cyclin D, and cyclin A gene transcription and increase expression of p21, p27 what leads to G2 cell cycle arrest[49].

3.2.6 Metabolic regulations related to estrogen signaling in breast carcinoma

Cancer cells are forced to adapt their features in order to survive. The modifications of cell metabolism are one of the key tasks in tumor progression. E2 regulates many of the metabolic pathways. Its signaling influence *e.g.* aerobic glycolysis, nucleotide, and amino acid synthesis, and glycerophospholipid metabolism.

The changes are mediated by ERα via several pathways that include the increase in production of metabolic intermediates for biosynthesis and consequently support proliferation[59].

Glycolysis is E2 dependent metabolic pathway that is regulated via membrane-initiated ER activation of PI3K-Akt signaling pathway leading to the upregulation of hexokinase activity and glycolysis[60]. The promoter of the gene for fructose-2,6-biphosphatase 3 (PFKFB3) contains ERE and is controlled by the ER. E2 upregulates the protein's synthesis that leads to the increased levels of fructose 2,6-bisphosphate, the product of PFKFB3 enzyme, and glucose uptake. Thanks to these processes, glycolysis is promoted[61].

The usage of metabolism is regulated regarding the current conditions. In conditions with high levels of glucose, BC cells use preferably glucose metabolism that is supported by E2 signaling. The signaling pathway is mediated by PI3K/Akt activation and stimulates the increase in glucose uptake as well as the expression of the genes from the glycolytic pathway. TCA cycle is inhibited due to the decreased pyruvate dehydrogenase (PDH) activity leading to low citrate concentrations. The activity of the PDH enzyme is important for the transformation of glucose to pyruvate in the TCA. E2 signaling is also responsible for the inhibition. If the cell finds itself in low glucose conditions, the opposite response follows. PDH enzyme activity increases leading to TCA cycle stimulation while glycolysis is inhibited. All these operations are controlled via E2 signaling pathways, although different kinases are used. In lack of glucose, PDH activity is stimulated by 5' AMP-activated protein kinase (AMPK), on the other hand, AMPK activity is inhibited by AKT action in high glucose concentrations[62].

The common feature of cancer cells is the increase of glutaminolysis compared to the normal cells. There is also a difference between ER- (negative) and ER+ (positive) BC cells in the manner of glutaminolysis[63]. Glutamine as a major amino acid in the blood and the major source of nitrogen is also connected to the cellular production of ATP by anaplerosis reaction of TCA as well as to the usage as a building block for protein and nucleic acid synthesis and antioxidant capacity[5].

The higher growth rate is ensured via the effect of E2 on BC cells leading to increased consumption of glucose and glutamine; the lactate levels and PPP flux increase. Glucose is used for both energetic and precursors gain. Glutamine is preferentially used as a biosynthetic precursors source instead of oxidizing in mitochondria. The PPP flux produces NADPH that is used in biosynthesis. Some glutamine is also used for FA synthesis. Also, the increase in glutamine consumption leads to a higher concentration of lactate in aerobic conditions[64]. BC cells also change serine/glycine biosynthesis and metabolism of FA that helps the cancer cell in surviving and proliferation[5].

The previously mentioned protooncogene c-Myc has also a different task in the reprograming glutamine metabolism. c-Myc enhances the transcription of the gene belonging to the glycolytic cascade[15].

Malate-aspartate shuttle is almost irreversible. Just a small amount of cytosolic malate and NADH are transferred to the mitochondrion. NADH is transferred from glycolysis in small doses to the mitochondrion. Nevertheless, most of NADH stays in the cytosol; NADH/NAD+ ratio increases and this ratio shifts lactate dehydrogenase equilibrium towards lactate[64].

In summary, both aerobic glycolysis and lactate production are amplified. The excretion of malate from mitochondrion is preferred instead of mitochondrial consumption. That leads to high cytosolic levels of both, aspartate and citrate, the precursors for *de novo* biosynthesis of nucleosides, proteins, lipids, and cholesterol that are needed for the cell growth[64].

Rapidly dividing cells must also replicate their DNA. The excessive synthesis of DNA requires supplementation of the nucleotide precursors. The nucleotides are synthesized in one-carbon metabolism that needs folate molecules for proper functioning. Folate-synthesis and one-carbon metabolism are regulated by E2. Folate metabolism has two options to fuel one-carbon metabolism; mitochondrial or cytosolic folate pathway. E2 selectively activates the mitochondrial one [65].

The polyamine and *de novo* purine synthesis are also upregulated by E2 due to the regulation of the important proteins involved in this pathway. These two are essential for proliferation and migration of ER+ (positive) BC cells[65].

The formation and maintenance of vesicular membranes are crucial for proliferating cancer cells. E2 supports glycerophospholipid metabolism pathway through the changes in choline metabolism level. The levels of phosphocholine (PCho) decrease and the levels of phosphatidylcholine (PtdCho) increase. E2 signaling in BC cells stimulates choline phosphotransferase 1 (CHPT1) that affects the levels of PtdCho. The rate-limiting enzyme in PtdCho synthesis is CCT (CTP: phosphocholine cytidylyltransferase) which expression is upregulated in BC. The CCT is considered a metabolic hallmark of cancer. E2 supports the synthesis of precursors for subsequent biosynthesis. In E2 signaling cascade, the induction of PtdCho production is upregulated thanks to the changed expression of CHPT1 and the increase in precursor synsthesis[59].

PCho/GDP ratio is associated with malignant transformation. Lowered levels of CHPT1 leads to the reduction of the ratio and in consequence, to the decrease of proliferation and growth of the transferring cells. CHPT1 is used in an early stage of metastasis. The upregulation of CHPT1 leads to the higher levels of PtdCho and that supports the cell membrane synthesis[59].

The metabolic pathway that supports proliferation via synthesis of amino acids is also beta-alanine metabolism. This pathway is increased in BC cells compared to normal tissue. ABAT (4-Aminobutyrate aminotransferase) protein expression is up-regulated in ER+ BC. This protein action leads to the supplementation of TCA by the synthesis of acetyl-CoA[63].

In BC cells estrogen stimulates expression of ornithine decarboxylase, the enzyme involved in the synthesis of polyamines[66].

There are also alternations in the cancer stem cells (CSCs) energy metabolism. These cells are more glycolytic than differentiated tumor cells. More glycolytic cells are usually more aggressive and have increased multidrug resistance. Such stimulation causes the induction of anaerobic glycolysis and oxidative phosphorylation is slowed down. Stimulating of ERβ leads to apoptosis in adherent BC cells through the mitochondrial way, although CSCs are not fully dependent on oxidative respiration, therefore, CSCs may benefit from ERβ signaling due to enhanced glycolysis[46].

Lots of these changes could explain resistance to hormone treatment in breast cancer[65]. The differences in tumor metabolism could be used as a diagnostic target or the target of the therapy[67].

4. Resistance to hormone treatment in breast carcinoma

BC is a hormone responsive disease and ER, as an important regulator in the tumor growth, is frequently used as a target of hormone treatment. The cancer response to the endocrine therapy correlates with the levels of PR and ER; their expression is linked together[57]. About 70-75 % of invasive BC cells express ER[59]. The advantage of the treatment is that these two receptors are rare in normal breast tissue[57].

Endocrine agents perform the therapeutic effect by changes in angiogenesis, differentiation, invasion, and metastasis. The effectiveness of endocrine therapy depends on the efficiency of the induction of cell proliferation arrest[68]. Nowadays, there are two strategies in anti-hormone therapy. First, long-term treatment inhibits estrogen-stimulated growth by blocking of tumor ER. Second, the treatment blocks the estrogen biosynthesis[69].

4.1 Selective estrogen receptor modulators (SERMs)

Molecules belonging to this group of pharmaceuticals are used for their ability to antagonize ER. ER-binding SERM molecule is incapable of making the conformational changes required for recruitment of the coactivators and corepressors[70]. Antiestrogen competes with E2 for the binding spot of the ER.

Tamoxifen (TAM) is an amino-ether derivate of polycyclic phenol[71] metabolized in the human body. The metabolization changes molecular features, but it is not required for TAM response[72]. However, metabolism of TAM molecules leads to different affinity to ERα of arose molecules. Some of the metabolites have higher and some lower affinity to ERα than TAM[73]. TAM action is mediated by ER; there is a significant correlation between the ER levels and the response to TAM. The loss of ER usually signifies the resistance to TAM and is a major reason for developing *de novo* resistance[74].

The antiestrogen induces the delay in the replenishment of the cytosolic ER; the ER-antiestrogen complex interacts differently with chromatin acceptor sites than the ER-E2 complex[72].

Antiestrogens selectively inhibit proliferation of the ER expressing BC cells. Treated cells are arrested in the G0-G1 phase of the cell cycle. This arrest is reversible by E2. Antiestrogens suppress E2 mediated stimulation of plasminogen activator activity. Its increase leads to the elevation of metastatic potential of tumor cells[72]. TAM blocks an estrogen-induced increase in PR[75].

4.2 Selective estrogen receptor downregulators (SERDs)

Resistant tumors to the TAM therapy still respond to another hormone treatment, *e.g.* SERDs and/or aromatase inhibitors (AIs). This suggests ER dependence of these tumors[76]. Fulvestrant (FVS) is SERD prescribed to postmenopausal women who developed resistance to TAM or AIs treatment and have ER+ BC[77]. The steroidal drug FVS is the pure ER antagonist with ERα affinity 100-fold greater than TAM has. Moreover, ER-binding FVS inhibits the dimerization and signaling cascade, so it cannot be started[78].

FVS as the pure anti-estrogen has no known agonistic effect mediated by ER. The conformational changes dictated by the bound of FVS that promotes the receptor to degradation. This results in downregulation of protein and to depletion of ER transcriptional activity. ER degradation induces the reduction of the ER-regulated proteins (*e.g.*, PR, pS2, cell survival protein Bcl-2) and the reduction of proliferation. FVS treatment leads to almost complete inhibition of gene transcription. FVS treatment may lead to the ER-negative phenotype[79].

TAM blocks the activity of AF-2, AF-1 activity remains the same. Also, TAM acts partially as an agonist. On the other hand, after FVS binding to ER, the conformational change occurs, and the change disrupts both AF-1 and AF-2 related transcriptional activity[80]. FVS blocks nuclear localization and inhibits dimerization as well[81]. Altogether, it leads to the inhibition of E2 signaling mediated by ER[82].

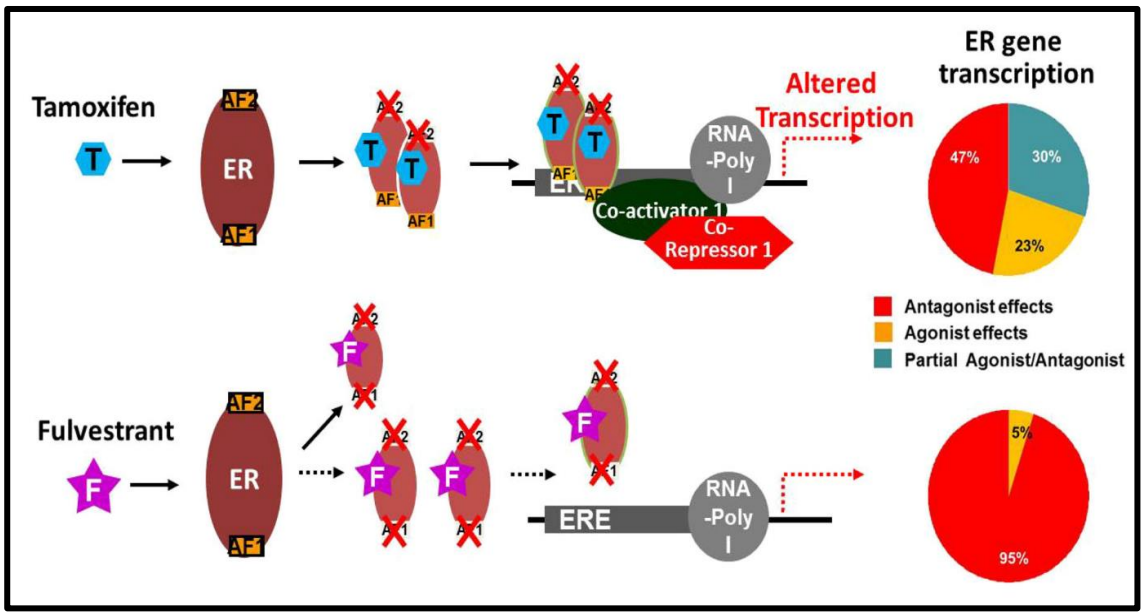

Fig. 10. Different action of TAM and FVS.

Tamoxifen (T), belonging to the SERM family, inhibits only activation function 2 (AF-2). Activation function 1 (AF-1) is still functional and estrogen receptor (ER) is still able to affect the cell growth by its signaling and alternations in transcription. T also has a partial agonist effect on ER gene transcription. On the other hand, fulvestrant (F), from the SERD family, inhibits both AF-1 and AF-2. F has a lightly agonistic effect on the gene transcription. Taken from [82].

4.3 Aromatase inhibitors (AIs)

Two types of AIs are used as a treatment; steroidal and non-steroidal. The steroidal or so-called type I inhibitors are structurally like androstenedione, the substrate for aromatase enzyme. This substance binds to the enzyme and is converted to another intermediate that causes irreversible enzyme inhibition by its binding. Type II, the non-steroidal AIs, competes for the enzyme with its original substrate[83]. In summary, their effect lies in the inhibition of the enzyme that converts androgens to estrogens called aromatase[84].

E2 is mainly synthesized in postmenopausal woman body via the extraglandular conversion of the androstenedione (estrogen precursor) to estrone by aromatization. AIs stops the process of aromatization and inhibits the E2 production[85].

The function of anti-estrogens lies in obstruction of ER-mediated pathways, however, AIs block also ER and overall the production of E2 is significantly lowered. The receptor-independent E2 effect is also inhibited. The E2-independent effect lies in the metabolized E2 that can generate oxygen free radicals that cause DNA damage or other E2 metabolites causing the DNA alterations[86].

4.4 Mechanism of resistance to hormonal treatment in breast carcinoma

Hormone therapy brings a significant improvement in BC outcomes. Still, the clinical problem appears due to the development of resistance to endocrine therapy in treated cells. Tumor as an evolving process response to the hormonal treatment, nevertheless, resistance can be developed. The resistance exists in two forms: a) *de novo* (existing before treatment) or b) acquired (developed during therapy).

The acquired resistance can be triggered in many ways. One of them is based on a mutation in the gene for ER; called *ESR1*. Mutations in *ESR1* lead to receptor-ligand independence and reduce response to TAM and FVS in BC. The lack of estrogen, a strong selective pressure, results in the selection of the mutated cells in the *ESR1* gene, resulting in estrogen-independent growth. This process leads to the adaptation to E2 deprivation *in vitro*. Endocrine resistance is conditioned by mutations in *ESR1* and other signaling pathways[87].

Another option for the cell to escape estrogen-dependence is via activation of fibroblast growth factor receptor (FGFR) 3. Activation of this receptor leads to the promotion of the TAM resistance[88]. FGFR2 is also involved in the development of endocrine resistance. FGFR2 signaling pathway governs the ER function and affects the action of TAM. This type of resistance is linked together with microenvironment stimuli that start the signal mediated via FGFR2[89].

E2 pathway is very complex, so are the pathways to resistance. One of the ways is the alteration of the enzyme that metabolizes the drug to its more active form (endoxifen). The mutation in such an enzyme slows down the enzymatic activity to the complete inhibition and results in a poorer outcome for the patients. ERα serves as a primary target of endocrine therapy. It is known that tumor lacking ERα cannot

a response to TAM therapy. The complete protein loss is not necessary. The mutation in the ER gene is enough to cause functional ER-negative phenotype in the tumor.

The alteration of co-regulatory protein in ER action also plays a role in the endocrine resistance. Co-activators and co-repressors make a complex with ER and induce transcriptional changes in this pathway. TAM typically induces the action of co-repressors. Therefore, the altered expression of the co-regulator genes may cause resistance. Specifically, overexpression of co-activators leads to TAM resistance due to the overexpressed co-activator proteins ability of the enhancement of TAM agonistic activity.

The overexpression of pro-oncogene HER2 and EGFR are connected to the poorer response to the hormonal therapy in ER+ BC cells[90,91].

The mentioned tumor suppressor activity of ERβ signaling is also the reason of endocrine resistance. Alteration in ERβ expression is involved in endocrine resistance.

The endocrine adaptation has a big role in the resistance of pre-menopausal women treated with TAM. The levels of estrogen are significantly higher and inhibit saturation of ER with TAM[92]. The co-activator AIB1 is very important in signaling leading to BC cell growth. Moreover, AIB1 acts as an enhancer of the agonistic activity of TAM. This may lead to resistance[93].

Also, the cell can develop TAM-stimulation features. These tumor cells grow in the response to either TAM or E2[94].

5. Conclusions

Breast cancer is an oncological disease that includes various phenotypes that are difficult to classify into the subgroups. The heterogeneity of phenotypes ensures the large variability of the cells.

The leading force of BC progression is stimulated via the estrogen receptor. The E2 signaling changes cell behavior to rapid progression and invasiveness.

BC cells are characterized, as most of the cancer cells in general, by alterations in the metabolic pathways. These changes are distinctive for the cancer cells and differ from normal cells of a human body. Cancer cells can preferentially use other energy sources than normal cells.

The plastic metabolism results from the sorting to BC subtypes. Cell metabolism also depends on other things: cell localization, nutrition concentration, and the metastatic phase.

Nowadays, there is a treatment that is effective in specific BC subgroups, the ER-positive tumors. The hormone treatment is based on the inhibition of estrogen signaling in tumor cells, which can be achieved by the inhibition of ER signaling via binding of an antagonist or by the inhibition of estrogen synthesis. About 30-40 % of postmenopausal breast cancer women respond to the hormone manipulation[95].

However, the treated tumors can develop resistance. The resistance can evolve in different ways, but the mechanism has not been fully discovered yet. To avoid the resistant tumor progression, it is important to find the new targets for cancer therapy.

BC cannot be considered as a single disease entity. The determination of BC subtype is conditional for the right selection of therapy.

Different metabolic requirements of cancer cells can be used in treatment. The therapy target must be exclusive for the cancer cell to decrease the toxicity the treatment may cause. Breast cancer is considered a metabolic disease. That is why the metabolic drug target can be found among the metabolic alterations in BC cells in metabolic pathways that are critical for BC proliferation. For example, in one-carbon folate metabolism that is steered via the mitochondrial pathway in BC cells. Also, *de novo* purine synthesis pathway that can be used in treatment targeting[65]. CHPT1, the important enzyme in choline metabolism, is overexpressed in BC cells and it is another possible target[59]. Another intervention site of the drugs can be found among the enzymes from PPP[64] and glutaminolysis[63,64]. There are also drugs targeting glycolysis, fatty acid synthesis, HIF signaling[63].

A better understanding of cancer cells and their unique features can be used in both the cancer therapies and the detection of the tumor.

6. List of abbreviations

AF-1	Activation function 1
AF-2	Activation function 2
AIs	Aromatase inhibitors
AMPK	5' AMP-activated protein kinase
ASCT2	Glutamine transporter
BC	Breast cancer
BM	Basal membrane
CHPT1	Choline phosphotransferase 1
CSC	Cancer stem cell
CTC	Circulating tumor cell
DBD	DNA binding domain
DCIS	Ductal carcinoma *in situ*
DRC	DNA repair capacity
E2	17-β-estradiol
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
EndMT	Endothelial-mesenchymal transition
ER-	Estrogen receptor negative
ER	Estrogen receptor
ER+	Estrogen receptor positive
ERE	Estrogen responsive element
FA	Fatty acid
FGFR	Fibroblast growth factor receptor
FVS	Fulvestrant
GLS	Glutaminase
GLUT	Glucose transporter
HIF	Hypoxia-inducible factor
HR	Homologous recombination
IDC	Invasive ductal carcinoma
IS	Immune system
LBD	Ligand binding domain
LCIS	Lobular carcinoma *in situ*
MAPK	Mitogen-activated protein kinase
MET	Mesenchymal-epithelial transformation
NF-κB	Nuclear factor κB
NLS	Nuclear localization signal
NO	Nitric oxide
NOD-like	Nucleotide-binding oligomerization domain-like
OXPHOS	Oxidative phosphorylation
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PFKFB3	Fructose-2,6-biphosphatase 3
PI3K/Akt	Phosphoinositide 3-kinases/protein kinase B
PPP	Pentose phosphate pathway
PR	Progesterone receptor
SERDs	Selective estrogen receptor downregulators
SERMs	Selective estrogen receptor modulators
TAM	Tamoxifen
TCA	Tricarboxylic acid cycle
TNBC	Triple-negative breast cancer
TNF-α	Tumor necrosis factor alpha

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